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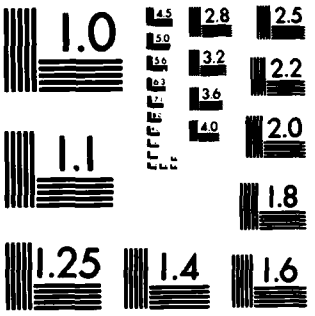

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Total Extractable Tin Measurement in Complex Matrices

An Evaluation

Carol A. Dooley
Giti Vafa

NAVAL OCEAN SYSTEMS CENTER

San Diego, California 92152-5000

F. M. PESTORIUS, CAPT, USN
Commander

R. M. HILLYER
Technical Director

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Direct measurement by GFAAS of organic extracts for extractable tin results in a concentration value highly dependent upon both sample composition and the standard used for the calibration. Measurement of these extracts after washing with NaOH and using Bu_3SnCl as a standard, however, results in a reliable measurement of Bu_3Sn in the extract.

Gas chromatography after derivatization of the butyltins with pentyl magnesium bromide and flame AAS after the formation of hydride derivatives were also used to measure concentrations of butyltins in organic extracts. Provided the sample for hydride derivatization is highly diluted to prevent organics interference, good correlation between the two methods is obtained for all butyltins and for Bu_3Sn measured by GFAAS after the NaOH wash.

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SUMMARY

The measurement of butyltins in tissues has become important to evaluate the uptake and food chain accumulation potential of tributyltin antifouling leachates. The work described in this report evaluates the total solvent extractable tin measurement for tissues and compares several methods for determining butyltin concentrations.

Direct measurement by graphite furnace atomic absorption spectrophotometry (GFAAS) of organic extracts for extractable tin resulted in a concentration value highly dependent upon both sample composition and the standard used for the calibration. Measurement of these extracts after washing with NaOH and using Bu_3SnCl as a standard, however, resulted in a reliable measurement of Bu_3Sn in the extract.

Gas chromatography after derivatization of the butyltins with pentyl magnesium bromide and flame AAS after the formation of hydride derivatives were also used to measure concentrations of butyltins in organic extracts. Provided the sample for hydride derivatization was highly diluted to prevent organics interference, good correlation between the two methods was obtained for all butyltins and for Bu_3Sn measured by GFAAS after the NaOH wash.

CONTENTS

ILLUSTRATION

TABLE

BACKGROUND

The Marine Environment Branch at Naval Ocean Systems Center is taking part in several related projects that require measuring organotin compounds in water, sediments, and tissues. Generally, the methodology to separate and measure low concentrations of these compounds in seawater by hydride derivatization/flame atomic absorption spectrophotometry is well developed (Valkirs et al., 1985). In certain instances, namely for sediments and tissues, the total solvent extractable tin is measured and reported for baseline studies and laboratory bioaccumulation studies. We needed to obtain a measure of the toxic species tributyltin (Bu_3SnX), where X is a labile anion dependent upon its environment, since total extractable tin could obviously encompass any organic soluble tin. We found that gross error can occur when a determination of total tin is made directly on an organic extract.

Determination of Bu_3Sn can be done by gas chromatography (GC) (Mueller, 1984; Burns et al., 1980, 1981), but the sensitivity is low and large numbers of samples would require a prohibitively long time. M&T Chemicals Incorporated (1979) has developed a multiple extraction method to determine the various species of tin in biological samples by graphite furnace atomic absorption spectrophotometry (GFAAS); while the whole procedure is lengthy and complex, the first step quantitatively separates the Bu_3Sn from other compounds. During a test of this method, we made the observations reported here.

METHODS AND MATERIALS

Oysters exposed to organotin compounds for 75 days in a laboratory test were shucked, blotted, and ground in a Tissumizer (Tekmar Instruments). Fifteen grams of wet tissue were stirred 10 minutes with 15 ml 1:1 aqueous HCl, then extracted for 4 hours with 50 ml of pesticide grade dichloromethane (Burdick & Jackson) on a rotary shaker. Duplicate 3.0 ml aliquots of CH_2Cl_2 extracts were spiked with 0.1 ml of 0.48 mg/ml Bu_3SnCl (Aldrich Chemical Company) in ethanol, 0.1 ml of 0.45 mg/ml Bu_2SnCl_2 (Aldrich Chemical Company) in ethanol, or both. A blank consisting of 3 ml CH_2Cl_2 was also prepared. One of each set was extracted with 3-percent aqueous NaOH for 30 seconds using a Vortex mixer. Aliquots of the organic layer were collected for GFAAS analysis, GC, and hydride derivatization/flame atomic absorption spectrophotometer (AAS) analysis.

We estimated the total solvent extractable tin using a Perkin-Elmer Model 5000 AAS equipped with an HGA-500 graphite furnace and an AS-40 autosampler. Aliquots of tissue extracts were diluted as necessary with methylisobutyl ketone (MIBK). The quantity of total tin was determined using standard additions of a freshly prepared tin AAS standard (5000-ppm organic tin salt in an organic matrix, Chemplex) in MIBK and tributyltin chloride (Aldrich Chemical Company) or dibutyltin dichloride (Aldrich Chemical Company) in 95-percent ethanol to prepare the calibration curve.

Derivatized butyltin compounds were determined, qualitatively and quantitatively, using a Varian 2100 gas chromatograph equipped with a hydrogen flame ionization

detector (FID). Samples were injected directly onto a 6-foot glass column packed with 3-percent OV-17 on Chromosorb W(HP) 80/100 mesh. Injector and detector temperatures were 170°C and 250°C, respectively. Gas flow rates of 30 ml/min for the hydrogen and helium carrier gas and 300 ml/min for the air were used. The oven was programmed from 100°C to 150°C at 10°C/min, after an initial 0.5-min hold. For analysis of tissue extracts, the oven temperature was increased to 200°C after 20 min. A Shimadzu CR3A Chromatopac was used for data collection.

Tissue extracts (0.5 ml) were evaporated to dryness and redissolved in 0.5-ml hexane prior to derivatization for gas chromatographic analysis. Hexane extracts were reacted at room temperature with a measured excess of the Grignard Reagent, 1.7 M pentylmagnesium bromide in diethylether (Alfa Ventron) for 20 minutes (Maguire & Huneault, 1981; Meinema et al., 1978). Excess Grignard Reagent was destroyed with 1N H_2SO_4, and hexadecane was added as an internal standard. Standards in hexane were prepared similarly from tributyltin chloride and dibutyltin dichloride.

For hydride derivatization/flame AAS measurements, 1.0-ml aliquots were evaporated to dryness, then redissolved in 2.0 ml of 95-percent ethanol. Some samples were further diluted as noted. 50-µl aliquots, diluted as necessary, were added to 500 ml of seawater in a reaction vessel. After adjusting the pH to 5-5.5 with 2N acetic acid, the sample was purged with helium at 40 ml/min. Five ml of four-percent $NaBH_4$ in 1-percent aqueous NaOH was then added, and the solution was purged for 5 minutes. The butyltin hydrides that are formed are carried into a quartz U-tube packed with 3-percent OV-1 on Chromosorb W(HP) 80/100 mesh and are immersed in liquid nitrogen. After purging, the U-tube is removed from the liquid nitrogen trap to permit volatilization of SnH_4 and $BuSnH_3$, then placed sequentially in a 50°C water bath and a 150°C oil bath to volatilize Bu_2SnH_2 and Bu_3SnH, respectively. Standards consisting of tributyltin chloride and dibutyltin dichloride in ethanol are prepared and treated in the same manner. The compounds are burned as they are eluted in an air/hydrogen flame and measured using a Buck Model 200 AAS at a wavelength of 286.3 nm.

RESULTS

Figure 1 shows the data from a previous experiment where the amount of total extractable tin and that portion identifiable as Bu_3Sn (expressed as tin) are shown as a function of time (Dooley, 1986). Total extractable tin was measured by GFAAS, and Bu_3Sn was measured by GC. The oysters (*Crassostrea virginica*) were first exposed to a constant level of Bu_3Sn, then removed to clean seawater after 60 days. In this controlled laboratory experiment, the amount of Bu_3Sn measured paralleled the amount of total extractable tin measured as long as a constant influx of Bu_3Sn was maintained. The values diverged when Bu_3Sn became mobilized (depurated or metabolized) from the tissue when the animals were removed to a clean environment. Obviously, with animals from an unknown exposure, we cannot know that Bu_3Sn is the major contribution to the tin atomic absorption signal. For this reason, we tested the method of selective solvent recovery for Bu_3Sn.

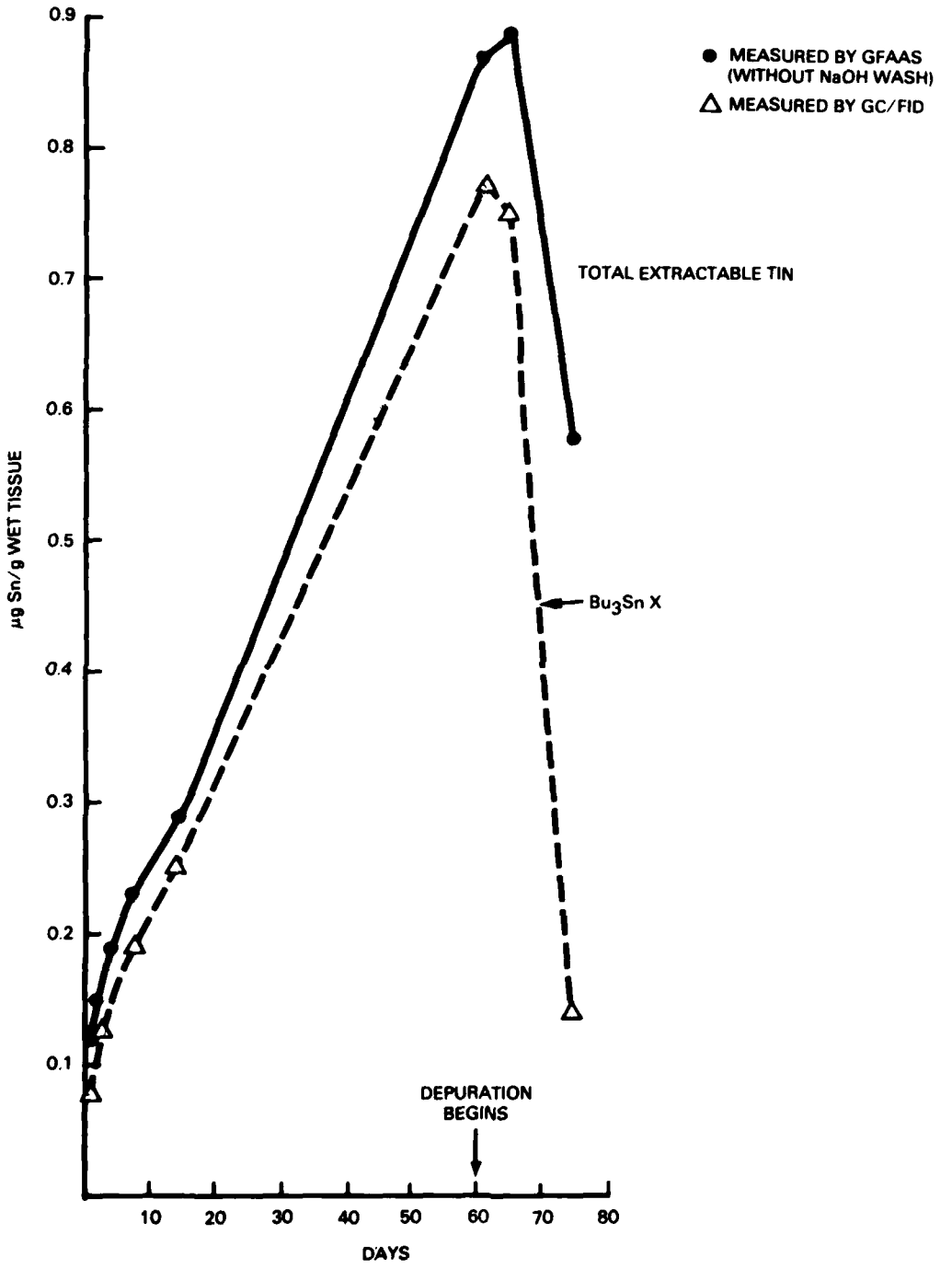

Figure 1. Total extractable tin and Bu_3Sn determined in oysters.

The results are best understood by referring to the list of experimental results in table 1. Column A shows the concentration of Bu_3Sn and Bu_2Sn added (as tin). In Column B, a + indicates that a counter extraction with NaOH was performed. NaOH should remove the Bu_2Sn and leave the Bu_3Sn in the organic layer. Column C shows the amount of tin measured using the purchased Chemplex 5000-ppm Sn AAS standard. Qualitatively, as predicted, the NaOH extraction appears to remove selectively the Bu_2Sn and the data appear consistent. However, the numerical data are highly inaccurate. Only ~50 percent of the expected amount of Bu_3Sn was measured and ~75 percent of the expected Bu_2Sn was measured. Columns D and E show the amount measured when the appropriate standard is used, i.e., Bu_3Sn and Bu_2Sn where only those compounds are known to be present. Thus, in samples 2 and 3, Bu_3Sn can be accurately measured, regardless of whether the NaOH extraction was performed. Standard additions, however, must be made to accommodate the changing matrix. An accurate measurement was obtained for sample 8, where extraction with NaOH removed the Bu_2Sn, but not for sample 7, which contained both compounds, with either the Chemplex AAS standard or the Bu_3SnCl standard. Bu_2Sn can be measured accurately in sample 5 using Bu_2SnCl_2 as a standard. Column F shows the amount of Bu_3Sn and Bu_2Sn measured by GC after derivatization with pentylmagnesium bromide. Good correlation between predicted and measured values is obtained.

Column G shows the dilution of the sample required to bring the peaks into the calibrated range for hydride derivatization/AAS measurement. The results are shown in Column H. This technique is still experimental for samples containing high organic loads and, thus, several features are observed. Accuracy of the determination is better with more dilute samples. This sensitive technique confirms the removal of Bu_2Sn from the system using NaOH, samples 6 and 8. At low dilutions, samples 1, 2, and 3, the measured values are low presumably due to a heavy organic load. Dilute samples, especially those treated with NaOH, which probably removed a great deal of organics, show good correlation with the added amount of compound. This shows both the high sensitivity of the method and the effect of organics on the system.

CONCLUSIONS

The values obtained by GFAAS for total extractable tin (without NaOH treatment) may be highly inaccurate regardless of the standard used to measure them. The interference is one that is not correctable by standard addition techniques. Total extractable tin measurements are of relative value only when the major source of contamination and compound sequestered is known to be primarily Bu_3Sn, e.g., in controlled laboratory experiments with organisms that cannot metabolize these compounds. However, for samples collected under natural conditions, the measured values may not be accurate and can only be considered as a screening device. Relative amounts should be valid. NaOH treated extracts provide a valid measure of Bu_3Sn.

Owing to the sensitivity and automation of GFAAS, this is a good method for measuring a large number of samples (e.g., in a monitoring program). GC does separate and measure accurately, but is not suitable for low concentrations without extensive preconcentration procedures. Hydride derivatization/flame AAS should be further developed, but empirically seems to face organics interference. Neither GC nor hydride derivatization/AAS are fast enough to accommodate large numbers of samples.

Table 1. Butyltins determined in CH_2Cl_2 extracts of oysters.

	µgSn/ml Added				µgSn/ml Measured							
Sample #					GFAAS			GC/FID		Hydride/AAS		
	Bu_3Sn	Bu_2Sn	Total	NaOH	AAS Std	Bu_3Sn	Bu_2Sn	Bu_3Sn	Bu_2Sn	Dil.	Bu_3Sn	Bu_2Sn
1	0	0	0		0.2	1.7	—	0.8	0.2	1	0.1	<0.1
2	0	0	0	+	0.1	1.0	—	0.7	NM	1	0.1	<0.1
3	5.9	0	5.9		3.1	5.8	—	—	—	1	3.0	0.1
4	5.9	0	5.9	+	3.1	5.9	—	6.4	NM	3	8.7	0.2
5	0	6.0	6.0		4.7	—	6.8	—	—	15	NM	3.3
6	0	6.0	6.0	+	1.0	—	0.5	—	—	15	NM	NM
7	5.9	6.0	11.9		5.8	24.8	—	7.5	8.2	5	3.1	3.9
8	5.9	6.0	11.9	+	3.7	5.8	—	6.2	NM	5	7.5	NM
Column ID	A			B	C	D	E	F		G	H	I

NM = not measurable
— = not measured

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